Plant Physiol. (1990) 94, 1048–1055
0032-0889/90/94/1048/08/$01.00/0

Received for publication March 27, 1990
Accepted July 2, 1990

Mechanism of Photosynthesis Decrease by *Verticillium dahliae* in Potato[1]

Robert L. Bowden*[2], Douglas I. Rouse, and Thomas D. Sharkey

Department of Plant Pathology (R.L.B., D.I.R.) and Department of Botany (T.D.S.), University of Wisconsin, Madison, Wisconsin 53706

ABSTRACT

Young, visually symptomless leaves from potato (*Solanum tuberosum*) plants infected with *Verticillium dahliae* exhibited reduced carbon assimilation rate, stomatal conductance, and intercellular CO_2, but no increase in dark respiration, no change in the relationship between carbon assimilation rate versus intercellular CO_2, and no change in light use efficiency when intercellular CO_2 was held constant. Therefore, the initial decrease in photosynthesis caused by *V. dahliae* was caused by stomatal closure. Errors in the intercellular CO_2 calculation caused by uneven distribution of carbon assimilation rate across the leaf were tested by $^{14}CO_2$ autoradiography. Patchiness was found at a low frequency. Low stomatal conductance was correlated with low leaf water potentials. Infection did not affect leaf osmotic potentials.

The potato early dying disease causes losses of 30 to 50% in many potato growing regions of the United States (18). *Verticillium dahliae* Kleb. is the most important pathogen of the potato early dying complex in Wisconsin (13). Vascular fungal pathogens such as *V. dahliae* cause water stress in host plants by decreasing the hydraulic conductance of the xylem (1, 16, 25). The extent to which reduced hydraulic conductance explains disease symptomology is controversial.

One of the early symptoms of early dying disease in potato is reduction of rate of photosynthesis (3, 4). This reduction can be caused by water-stress-induced stomatal closure, which limits the supply of CO_2. In addition, it is possible that the water stress, or toxins produced by the fungus, affect the biochemical reactions of photosynthesis directly. The separation of stomatal effects from direct biochemical effects can be made by comparing the response of photosynthesis to the estimated partial pressure of CO_2 inside the leaf (8). If the relationship between photosynthesis and C_i[3] is the same in the control and infected plants, then direct effects of infection on the biochemistry of photosynthesis can be ruled out, especially if the relationship is determined at several light levels. If differences are found between control and infected plants, these differences can be interpreted using biochemically based models of photosynthesis (9, 20).

Using this analysis can be complicated when stomata close unevenly across the leaf (6, 23). This patchiness has been observed in water-stressed plants (6, 21). The occurrence of patchy photosynthesis can be checked by autoradiography.

We have tested for patchy photosynthesis using autoradiography in control and *Verticillium* infected potatoes. We determined the relationship between photosynthesis and C_i at three light levels. Then we compared the relationship between stomatal conductance and water potential.

MATERIALS AND METHODS

Plant Culture

Axenically propagated, virus-free cuttings of potato (*Solanum tuberosum* L. cv Russet Burbank) were obtained from the University of Wisconsin seed potato program. Plants were grown in 20 L pots in large walk-in growth chambers under a 14 h photoperiod (0600–2000) with 25°C/15°C day/night temperature, 50% RH, and 550 μmol m^{-2} s^{-1} PAR at canopy height. Plants were automatically watered to excess four times per day with one-eighth strength Hoagland solution. In experiments 1 and 4, plants were grown in peat/vermiculite (1:1, v:v; bulk density 0.2) and in experiments 2 and 3, plants were grown in pasteurized (65°C, 30 min) Plainfield loamy sand (bulk density 1.4).

Inoculum Preparation

Inoculum was produced from pathogenic cultures of *Verticillium dahliae* which were isolated from potato. In experiment 1, eight cultures were grown on 10% strength Difco potato dextrose agar for 2 weeks. The agar containing the fungal colonies was homogenized in a blender, then mixed with peat-vermiculite at the rate of 1 mL/L of soil mix. The infested soil was dried and stored 5 months at room temperature prior to use.

In experiments 2 through 4, three isolates of *V. dahliae* from potato were grown on autoclaved rye grain at 20°C for 4 weeks. The infested grain was dried for 2 weeks at room temperature, then ground in a Wiley mill. Dried, ground rye

[1] Research was supported in part by Hatch Project 142–2500, Wisconsin Potato Industry Board, and U.S. Department of Agriculture Cooperative State Research Service Regional IPM grant 8702777.

[2] Present address: Department of Plant Pathology, Kansas State University, Manhattan, KS 66506.

[3] Abbreviations: C_i, CO_2 concentration (ppm) in the air spaces inside the leaf; A, net carbon assimilation rate; C_a, ambient CO_2 concentration; RuBPCase, RuBP carboxylase/oxygenase; RuBP, ribulose 1,5-bisphosphate; ppm, parts per million.

grain inoculum containing microsclerotia of *V. dahliae* was mixed by hand with the soil.

Soil samples were collected 3 weeks after planting, dried at room temperature for 2 weeks, then assayed by plating soil dilutions on PM selective medium as described by Nicot and Rouse (15). Estimated inoculum density for experiments 1 through 4 were 24, 680, 250, and 313 propagules per cm^3 of soil, respectively.

Gas Exchange Measurements

Young (approximately two-thirds expanded), nonsymptomatic leaves were selected for study from plants with symptoms of wilting and chlorosis on the older leaves. Measurement of A, stomatal conductance, and calculation of C_i were made with a LI-COR (Lincoln, NE 68504) LI-6200 portable photosynthesis system. Atmospheric pressure averaged 990 mb. Assimilation and stomatal conductance values represent the sum from both sides of the leaf.

CO_2 response curves (A–C_i curves) were obtained *in situ* between the hours of 0900 and 1400 in the walk-in growth chambers so that plants were disturbed as little as possible. A portion of the terminal leaflet of a young, symptomless, unshaded leaf was enclosed in the LI-6200 quarter-liter leaf chamber. The chamber was held stationary by a ring stand. The leaf was illuminated by a Sylvania 300W ELH bulb in a Kodak slide projector. Light level at the leaf was adjusted to 1500 ± 100 μmol m^{-2} s^{-1} by changing the distance between projector and leaf. Corrections were made for light attenuation by the chamber wall. During the 30 min equilibration period, the LI-6200 was operated in open mode and the C_a was 340 ± 5 ppm. Leaf temperature was held at 26 ± 1.0°C by changing the temperature of the walk-in growth chamber. The RH in the leaf chamber was maintained at 52 ± 2.0% by changing the humidity of the growth chamber and the proportion of air flow passed through magnesium perchlorate desiccant. For any given photosynthesis measurement, the change in RH was less than 1.0%. CO_2 response curves were started by changing the LI-6200 to closed mode at which time the CO_2 concentration began to be depleted by the leaf. The first measurement typically had a mean C_a of approximately 300 ppm. Measurements averaged over 10 s were taken at approximately 50 ppm intervals of C_a. Occasionally, the process was accelerated by momentarily switching the air flow through soda lime to remove some CO_2. In that case, extra time was allowed for the system to stabilize before taking the next reading. When ambient CO_2 reached about 50 ppm (*i.e.* the compensation point), the system was switched back to open mode and a puff of CO_2 was breathed into the chamber. The level was adjusted to about 1500 ppm by scrubbing with soda lime. The system was then switched back to the closed mode and readings were taken approximately every 200 ppm until the C_a was below 350 ppm. Response curves were interpolated to obtain assimilation rates at particular CO_2 levels. RuBP use rates were calculated according to the formula of Brooks and Farquhar (5). Treatment means were analyzed as a one-way ANOVA with SAS procedure GLM (19).

Autoradiographs

Immediately after the CO_2 response curve was complete, the system was switched back to open mode and the leaf was reequilibrated at C_a = 340 ± 5 ppm for 10 min. Afterward 350 ± 30 ppm $^{14}CO_2$ (0.1 mCi $mmol^{-1}$) was passed through the leaf chamber for 3 min. The leaflet was excised and immediately frozen beneath a block of aluminum cooled to the temperature of liquid nitrogen. The frozen leaf was placed directly against x-ray film and exposed for 1 week at −80°C. Some additional leaflets were exposed in a larger 1.8 L leaf chamber in order to make autoradiographs of the entire leaflet.

Reconstructed Light Response Curves

Plants were moved from the original growth chamber to a darkened growth chamber and CO_2 response curves were obtained at light levels of 1000, 400, and 200 μmol m^{-2} s^{-1}, in that order. The initial equilibration period was 30 min, and a 10 min reequilibration period was allowed between each light level. Afterward, the light was turned off and dark respiration was measured. Light response curves at C_a = 300 ppm were reconstructed by interpolating values from the A *versus* C_a response curves. Light response curves at C_i = 220 ppm were reconstructed from the corresponding A *versus* C_i response curves.

Water Potential Measurements

Water potentials were measured with a pressure bomb (model 3005, Soilmoisture Equipment Corp., Santa Barbara, CA 93105) following the general procedures of Gandar and Tanner (10). The terminal leaflet was sealed in an 8 by 15 cm plastic bag containing a damp piece of filter paper. The terminal leaflet and a 1 to 2 cm piece of petiolule were then immediately excised with a razor blade. The petiolule was placed through a custom-made silicon rubber (RTV-11, Gen-

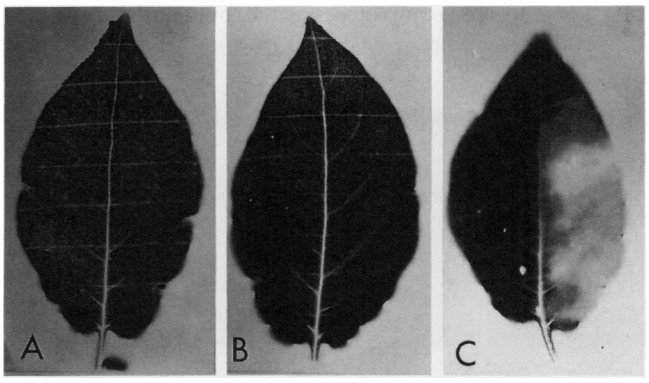

Figure 1. ^{14}C autoradiographs of A, uninoculated leaf; B, inoculated uniform leaf; and C, inoculated leaf with unilateral dysfunction from experiment 2 at 108 d after planting. The horizontal lines were caused by shadows of the supporting monofilament line.

eral Electric, Waterford, NY 12188) seal with the hole molded in the shape of a potato petiolule.

The pressure-volume curve technique described by Hinckley *et al.* (12) was used to determine the osmotic potentials of potato leaves. Terminal leaflets were sampled between 1200 and 1300.

Drought Stress Treatments

In experiment 1, four control plants and eight inoculated plants were watered as usual. Water was withheld from four uninoculated plants starting at 102 d after planting. Stomatal conductance and water potential measurements were taken from 4 to 6 young, unshaded leaves per plant on 3 d starting 2 d after cessation of watering. This experiment involved a single cycle of drought stress.

In experiments 2 and 3, water was withheld from four uninoculated plants starting at 86 and 96 d after planting, respectively. Thereafter, plants were watered with 800 mL distilled water whenever they began to wilt (every 3–4 d). Four uninoculated plants and eight inoculated plants were watered normally. Gas exchange and water potential measurements were taken starting two weeks after the drought stress was initiated.

RESULTS

Autoradiographs

Recent work (6, 23) has shown that uneven distribution of photosynthesis across the leaf (hereafter referred to as patchiness) can cause a bias in calculation of C_i. This then appears as a nonstomatal limitation to photosynthesis in the A to C_i curves. Fifty-five autoradiographs were prepared from inoculated and uninoculated leaves to test for patchiness. Representative autoradiographs are presented in Figures 1 to 3. All uninoculated leaves and most leaves from inoculated plants exhibited uniform photosynthesis across the leaf. One older leaf from an infected plant exhibited unilateral turgor loss and chlorosis and showed a matching unilateral decrease in photosynthesis in the autoradiograph (Fig. 1C). Another leaf (Fig. 2D) appeared completely normal, but felt slightly flaccid

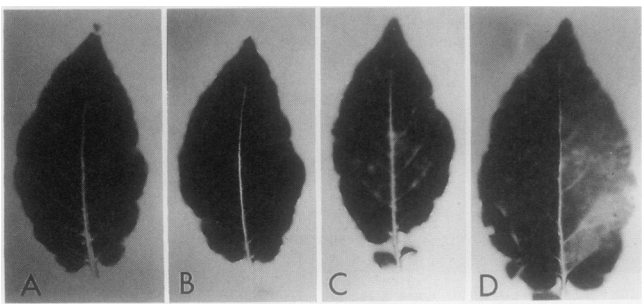

Figure 2. ^{14}C autoradiographs of A, uninoculated leaf; B, inoculated uniform leaf; C, inoculated leaf with patchy dysfunction; and D, inoculated leaf with unilateral dysfunction from experiment 3 at 118 to 120 d after planting.

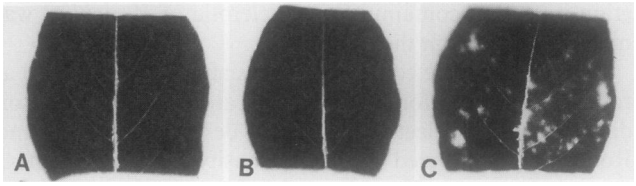

Figure 3. ^{14}C autoradiographs of A, uninoculated leaf; B, inoculated uniform leaf; and C, inoculated leaf with patchy dysfunction from experiment 4 at 72 to 76 d after planting. Autoradiographs appear truncated because leaves were bigger than the labeling chamber.

on one side. The autoradiograph revealed a unilateral dysfunction. Two normal-appearing leaves from inoculated plants (Figs. 2C and 3C) showed small (1–5 mm) spots with decreased assimilation rates. Only one leaf used for CO_2 response curves had detectable patchiness (experiment 4; Fig. 3C).

CO_2 Response Curves

CO_2 response curves obtained in experiments 3 and 4 were similar; therefore, only the results from experiment 3 are presented (Fig. 4). First, it was necessary to demonstrate that the sampled leaves from inoculated plants were diseased. Since stomatal conductance changed during the CO_2 response studies, comparisons were based on the first measurement after the equilibration period. In a closed system like the LI-6200, this measurement has a mean CO_2 concentration below ambient air levels. A mean C_a of 300 ± 2 ppm was chosen for comparison and values were interpolated from A to C_a curves if necessary. In both experiments, there was a significant effect of *Verticillium* infection on the assimilation rate, stomatal conductance, and C_i (Table I).

The initial slope of the A to C_i response curve is linearly related to the activity of RuBPCase (26). Infection had no

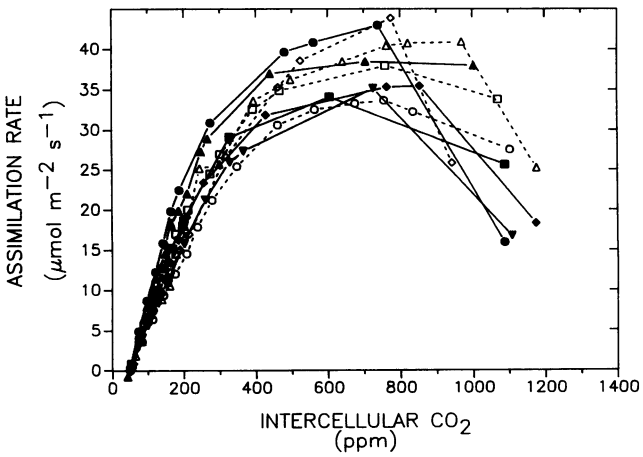

Figure 4. CO_2 response curves from experiment 3 at 118 to 120 d after planting. Each line represents a single leaf on a different plant. Closed symbols are inoculated, open symbols are controls.

Table I. *Means of CO_2 Response Curve Data from Experiment 3 at 118 to 120 Days after Planting and Experiment 4 at 72 to 76 Days after Planting*

All initial measurements were made at C_a = 300 ± 2 ppm from leaves equilibrated to saturating light and 340 ± 5 ppm CO_2 for 30 min.

Variable	Uninoculated	Inoculated	p-value[a]
Experiment 3			
Number of replicates	4	5	
Initial assimilation rate (μmol m^{-2} s^{-1})	19.8	16.6	0.040
Initial stomatal conductance (mol m^{-2} s^{-1})	0.49	0.23	0.009
Initial C_i (ppm)	209	164	0.006
Initial slope (dA/dC_i) (mol m^{-2} s^{-1})	0.118	0.136	0.187
Assimilation rate at C_i = 600 ppm (μmol m^{-2} s^{-1})	36.7	35.9	0.722
Experiment 4			
Number of replicates	4	4	
Initial assimilation rate (μmol m^{-2} s^{-1})	22.0	14.5	0.007
Initial stomatal conductance (mol m^{-2} s^{-1})	0.65	0.22	0.001
Initial C_i (ppm)	224	170	0.004
Initial slope (dA/dC_i) (mol m^{-2} s^{-1})	0.132	0.117	0.088
Assimilation rate at C_i = 600 ppm (μmol m^{-2} s^{-1})	33.3	30.1	0.086

[a] Probability that treatments are not different based on one-way ANOVA.

significant effect on the initial slope of the curves indicating that RuBPCase was unaffected by infection (Table I).

The assimilation rate at C_i = 600 ppm was used to test for RuBP regeneration limitations. For both treatments, assimilation rates at C_i = 600 were at least 1.5 times the assimilation rates at ambient CO_2 levels (Table I). In leaves from *Verticillium*-inoculated plants, RuBP use rates were increased between 25 and 27% at the higher CO_2 level (data not shown). This indicated that leaves from infected plants had excess RuBP regeneration capacity at ambient CO_2.

Above C_i = 600 ppm, behavior was unstable and characterized by reversed sensitivity to CO_2. Sharkey and Vassey (22) showed that this behavior is caused by inhibition of starch synthesis in healthy potato leaves. Since this triose phosphate utilization limitation occurs only at high C_i, it is not related to the *Verticillium*-induced decrease in photosynthesis.

The effect of *Verticillium* infection on photosynthesis could not be attributed to limitations in RuBPCase, RuBP regeneration, or triose phosphate utilization. Therefore the effect must be produced by a reduced supply of CO_2 caused by stomatal closure. The adequacy of this explanation can be tested by evaluating the assimilation rate of uninoculated leaves at the C_i exhibited by the inoculated leaves. The expected assimilation rate would be 14.2 and 15.2 μmol m^{-2} s^{-1} for experiments 3 and 4, respectively. This is consistent with the rates of 16.6 and 14.5, respectively, that were measured in the inoculated plants (Table I).

Reconstructed Light Response Curves

A to C_i response curves at three light levels for representative inoculated and uninoculated leaves are presented in Figure 5. The corresponding A to C_a curves were used to reconstruct light response curves at C_a = 300 ppm (Fig. 6A). At the high light level, assimilation rate, stomatal conductance and C_i were significantly decreased by *Verticillium* (Table II). At lower light levels, infection had no effect on assimilation rate. In order to remove stomatal effects, light response curves were reconstructed from A to C_i curves at C_i = 220 ppm (Fig. 6B). This correction removed the small treatment difference at the high light level and confirmed that stomatal closure accounts for the loss of light use efficiency. Infection did not affect leaf dark respiration rate (Fig. 6).

Stomatal Conductance *versus* Water Potential

The relationship between stomatal conductance and leaf water potential in experiment 1 is presented in Figure 7. Similar results were obtained in experiment 2. Leaves with water potentials below about −1.0 MPa appeared somewhat flaccid. The log transformation of stomatal conductance was used to linearize the relationship. In both experiments, stomatal conductance of leaves from inoculated plants was significantly ($P < 0.0001$) related to leaf water potential. In experiment 1 the R^2 was 0.783 and in experiment 2 the R^2 was 0.396.

To gauge whether stomata in infected leaves behaved normally, the relationship between stomatal conductance and leaf water potential was also studied in uninoculated plants. Low water potentials in uninfected plants were achieved by withholding water for various periods, while low water potentials in inoculated plants were caused by the disease. Plots of regression residuals showed evidence of a plateau at higher water potentials for the uninoculated leaves in experiment 1. Therefore, a segmented linear model was used and the plateau breakpoint was fitted with SAS (19) procedure NLIN to −0.44 MPa. In experiment 1, the slope of the uninoculated leaf regression line was significantly steeper ($P < 0.0001$). In experiment 2, the slopes were not significantly different ($P = 0.1043$), but the intercept of the uninoculated leaf regression line was significantly lower ($P < 0.0001$). In both experiments, drought-stressed leaves had lower stomatal conductances than leaves from *Verticillium*-infected plants for a given leaf water potential.

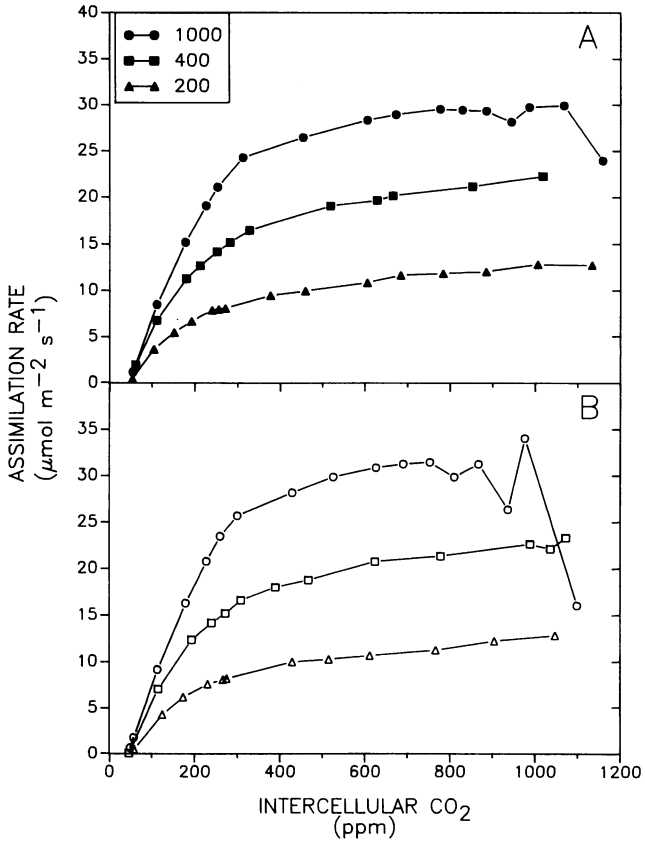

Figure 5. CO_2 response curves from experiment 4 at 85 to 87 d after planting at 1000, 400, and 200 μmol m^{-2} s^{-1} PAR. A, representative curve from inoculated plant; B, from uninoculated plant.

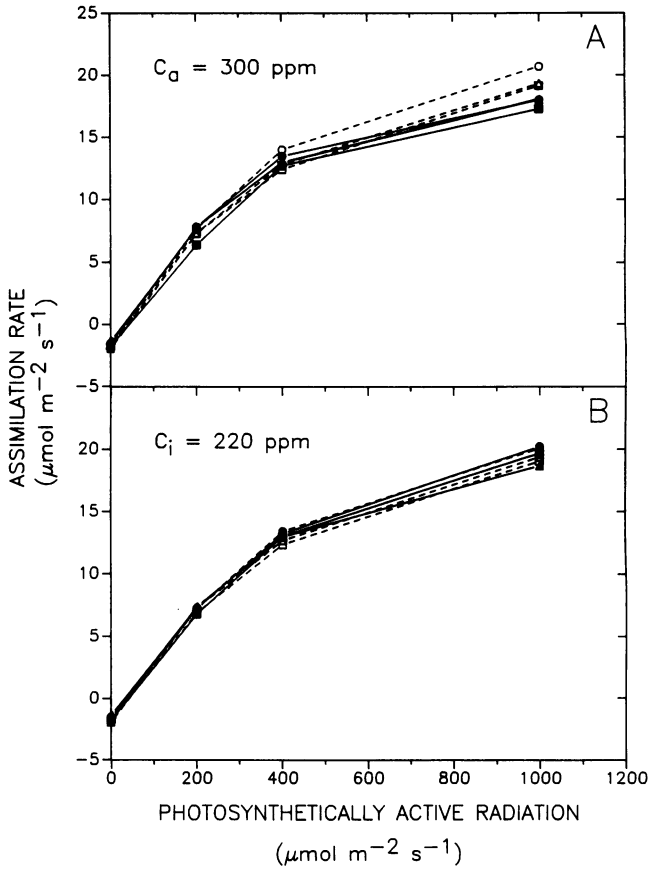

Figure 6. Reconstructed light response curves from experiment 4 at 85 to 87 d after planting. A, At ambient CO_2 of 300 ppm; B, at intercellular CO_2 of 220 ppm. Closed symbols are inoculated, open symbols are controls.

Osmotic Potentials

Differences in leaf osmotic properties were investigated as possible explanations for the difference in stomatal behavior. *Verticillium* infection had no significant effect on the osmotic potential at full turgor or the osmotic potential at zero turgor (Table III). In experiment 2 drought-stress caused a significant increase in osmotic potential at zero turgor of about 0.1 MPa. There was no significant effect on fully saturated osmotic potential and this might be explained by less elasticity in the drought-stressed leaves.

DISCUSSION

Young, symptomless leaves from potato plants infected with *V. dahliae* exhibited reduced photosynthesis, stomatal conductance, and C_i, but no change in the relationship between photosynthesis and C_i at three different light intensities. The effect of disease on the response of assimilation to light was removed by correcting for differences in C_i. Therefore, the initial decrease in photosynthesis in diseased leaves was caused by reduced CO_2 supply resulting from stomatal clo-

Table II. *Means from Reconstructed Light Response Curves from Experiment 4 at 85 to 87 Days after Planting*

All initial measurements were made at C_a = 300 ± 2 ppm from leaves equilibrated to 1000 μmol m^{-2} s^{-1} of PAR and 340 ± 5 ppm CO_2 for 30 min.

Variable	Uninoculated	Inoculated	P-Value[a]
Number of replicates	3	3	
Initial assimilation rate (μmol m^{-2} s^{-1})	19.7	17.8	0.024
Initial stomatal conductance (mol m^{-2} s^{-1})	0.56	0.35	0.011
Initial C_i (ppm)	223	200	0.024
Assimilation rate at C_i = 220 ppm[b] (μmol m^{-2} s^{-1})	19.5	19.5	0.959

[a] Probability that treatments are not different. [b] Interpolated from A to C_i curve at 1000 μmol m^{-2} s^{-1} PAR.

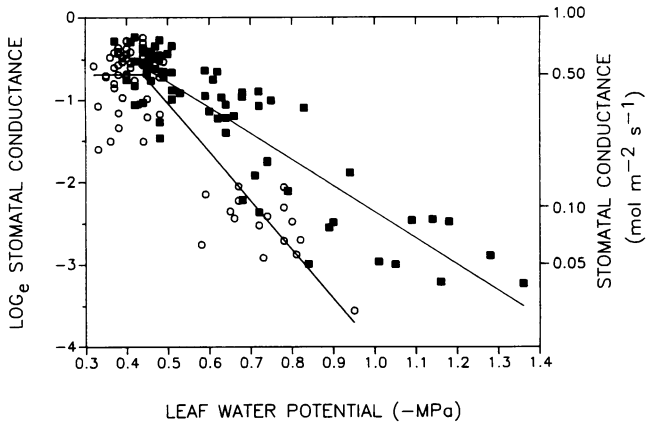

Figure 7. Relationship of natural log of stomatal conductance and leaf water potential in experiment 1 at 103 to 106 d after planting. Three to six leaves from four controls, four drought-stressed plants, and eight inoculated plants.

sure. There was no indication of direct effects of water stress or other pathogenic mechanisms such as toxins, enzymes, or hormones on the photosynthetic mechanism. Of course, as diseased leaves senesce, other effects such as chlorosis occur, but these are secondary.

The decrease in photosynthesis caused by *V. dahliae* was greatest in high light (Fig. 6; ref. 3). This relationship is partially explained with reference to Figure 5. The slope of the A to C_i curve between 200 and 250 ppm is much steeper at 1000 than at 200 μmol m^{-2} s^{-1} PAR. Therefore, a given change in C_i would produce a larger change in assimilation rate in high light. The difference in C_i between diseased and healthy leaves also tends to be higher under high light (3).

Stomatal closure was highly correlated with decreased leaf water potential (Fig. 7). Since we would not expect low stomatal conductance to cause water stress, a reasonable conclusion is that water stress is the cause of low stomatal conductance. Another possibility is that the relationship is coincidental. If stomata were closed by another mechanism (*e.g.* a toxin or hormonal effect), then there should be some leaves with low stomatal conductance and high leaf water potential, but no such leaves were found. All diseased leaves were under water stress and all water stressed leaves were diseased.

If *Verticillium* causes stomatal closure by inducing water stress, then mitigation of water stress should lead to stomatal opening. A humidity experiment proved that stomata in diseased leaves were capable of opening when the vapor pressure gradient between air and leaf was reduced (2). All the evidence is consistent with and fully accounted for by the hypothesis that reduced water supply was responsible for stomatal closure. Since diseased plants were always grown in moist soil, reduced water supply must have been caused by decreased plant hydraulic conductance.

An important facet of this study was identification of diseased leaves with low intraleaf variability. In addition to the C_i bias problem, patchiness may make determination of leaf water potential difficult. Potato leaves with unilateral wilt were observed in which the pressure bomb balancing pressure was different by as much as 0.2 MPa for the two sides of the same leaf (data not presented). These problems were avoided by using leaves from the systemic phase of disease rather than the local phase (4) and by checking homogeneity with autoradiographs for critical gas exchange studies.

Two types of patchiness were identified which may have different causes. The two autoradiographs exhibiting unilateral dysfunction (Figs. 1C and 2D) were both from older leaves from near the middle of the stem. One of them had visible unilateral chlorosis and turgor loss while the other appeared normal but felt a little flaccid on one side. This pattern can be explained by local dysfunction of the xylem on one side of the leaf.

It is more difficult to explain the dysfunctional spots seen in Figures 2C and 3C. Interestingly, these spots were undetectable by eye with visible light. The round, fairly discrete shapes are not consistent with local xylem dysfunction. Local dysfunction should produce dysfunctional areas with sharp proximal margins and diffuse distal margins caused by redistribution of water around the blockage. Spots could be caused by ABA (6, 23), or they could be related to small localized colonies of *Verticillium* in the leaves. In any case, spots affected a relatively small proportion of the leaf and such leaves were uncommon.

The results of the present study are not in agreement with several previous gas exchange studies involving fungal vascular pathogens. Mathre (14) reported a reduction in ability of isolated cotton chloroplasts from *Verticillium* infected leaves to perform the Hill reaction. This was interpreted as possible evidence for the involvement of a toxin. Tzeng and DeVay (24) found that the T9 strain of *V. dahliae* decreased cotton seedling stomatal conductance in greenhouse studies. They suggested that accelerated senescence, presumably caused by hormonal changes, was responsible for stomatal closure. Duniway and Slatyer (7) reported both stomatal and nonstomatal limitations to photosynthesis in tomato 15 d after inoculation with *Fusarium oxysporum*. Since the pathogen was confined to the major veins, and leaves were apparently not under water stress, they suggested that toxic products were translocated into the leaf blade from the veins. In a preliminary

Table III. *Osmotic Potentials of Inoculated, Uninoculated Unstressed, and Uninoculated Drought-Stressed Leaves, n = 4*

Treatment	Ψ_i[a]	Ψ^b_{100}	Ψ^c_0
Experiment 2			
Inoculated unstressed		−0.708 A[d]	−0.947 AB
Uninoculated unstressed		−0.756 A	−0.973 A
Uninoculated drought-stressed		−0.721 A	−0.872 B
LSD[e]		0.071	0.077
Experiment 3			
Inoculated unstressed	−0.628 B	−0.841 A	−1.021 A
Uninoculated unstressed	−0.295 A	−0.859 A	−1.019 A
Uninoculated drought-stressed	−0.793 C	−0.792 A	−0.931 A
LSD	0.110	0.099	0.098

[a] Initial leaf water potential when leaf was sampled (MPa). [b] Leaf osmotic potential at full turgor (MPa). [c] Leaf osmotic potential at zero turgor (MPa). [d] Means followed by same letter are not significantly different. [e] Fisher's protected least significant difference at P = 0.05.

report, Pennypacker *et al.* (17) described reductions in activity of RuBPCase in symptomless leaves from alfalfa infected with *V. albo-atrum*. This was deduced from the initial slope of the A to C_i curves.

In contrast to the present study, these studies purported to show that toxins, enzymes, hormones, or other unknown mechanisms play a significant role in either decreased photosynthesis or stomatal closure. These studies represent a variety of fungal wilt pathosystems which may represent different disease physiologies. However, the experimental procedures used in these studies may also provide an explanation. For example, patchiness could account for some of the reported effects in these studies.

In the Russet Burbank potato/*V. dahliae* system, the evidence is compelling that water stress caused by decreased hydraulic conductance accounts for all the gas exchange effects in symptomless leaves. However, this does not eliminate toxins, hormones, or enzymes as important pathogenic mechanisms. They may be crucial disease determinants which allow colonization of the xylem by the pathogen. However, there is no evidence that they have a direct effect on leaf gas exchange. If they affect gas exchange, it is most likely by means of decreased plant hydraulic conductance. They also might play a role in leaf senescence.

There are some interesting differences between *Verticillium*-induced water stress and drought-induced water stress in potato. In two experiments, leaves from inoculated plants had higher stomatal conductances than drought-stressed plants at equal leaf water potentials. This was not explained by osmotic adjustment by diseased leaves since osmotic potentials at full turgor were not significantly different between the three treatments. This is in agreement with the conclusions of Vos and Groenwold (27) that potato lacks the capacity for significant osmotic adjustment.

Vos and Groenwold (27) reported that drought sometimes slightly increased osmotic potentials in potato. Similar drought effects were seen in this study on osmotic potential at zero turgor (Table III). This shift would cause loss of turgor at higher leaf water potentials and so would partly explain the difference between inoculated and uninoculated leaves in Figure 7. However, the shift along the water potential axis in Figure 7 is on the order of 0.2 to 0.3 MPa which means that some of the variability is unexplained.

Evidence is accumulating that stomatal conductance in some plants decreases in response to substances produced in the roots of drought-stressed plants (11). If such a mechanism existed in potato, it would tend to close stomata of drought-stressed leaves at higher leaf water potentials than expected. The mechanism would not be triggered in *Verticillium*-infected plants because their roots would be wet. This hypothetical mechanism could explain the balance of the differences in Figure 7.

ACKNOWLEDGMENTS

Mr. Steve Vicen prepared the figures. Mr. Kevin Smith helped prepare inoculum and gave other technical assistance.

LITERATURE CITED

1. **Beckman CH** (1987) The Nature of Wilt Diseases of Plants. APS Press, St. Paul, Minnesota
2. **Bowden, RL** (1989) Effects of *Verticillium dahliae* on gas exchange and water relations of potato. PhD thesis, University of Wisconsin-Madison
3. **Bowden RL, Rouse DI** (1990) Effects of *Verticillium dahliae* on gas exchange of potato. Phytopathology **80:** (in press)
4. **Bowden RL, Rouse DI** (1990) Chronology of gas exchange effects and growth effects of *Verticillium dahliae* infection in potato. Phytopathology **80:** (in press)
5. **Brooks A, Farquhar GD** (1985) Effect of temperature on the CO_2/O_2 specificity of ribulose-1,5-bisphosphate carboxylase/oxygenase and the rate of respiration in the light. Estimates from gas exchange measurements on spinach. Planta **165:** 397–406
6. **Downton WJS, Loveys BR, Grant WJR** (1988) Stomatal closure fully accounts for the inhibition of photosynthesis by abscisic acid. New Phytol **108:** 263–266
7. **Duniway JM, Slatyer RO** (1971) Gas exchange studies on the transpiration and photosynthesis of tomato leaves affected by *Fusarium oxysporum* f.sp. *lycopersici*. Phytopathology **61:** 1377–1381
8. **Farquhar GD, Sharkey TD** (1982) Stomatal conductance and photosynthesis. Annu Rev Plant Physiol **33:** 317–345
9. **Farquhar GD, von Caemmerer S** (1982) Modelling of photosynthetic response to environmental conditions. *In* OL Lange, PS Nobel, CB Osmond, H Zeigler, eds, Encyclopedia of Plant Physiology, New Series. 12B. Springer-Verlag, New York, pp 549–587
10. **Gandar PW, Tanner CB** (1976) Potato leaf and tuber water potential measurements with a pressure chamber. Am Potato J **53:** 1–14
11. **Gollan T, Passioura JB, Munns R** (1986) Soil water status affects the stomatal conductance of fully turgid wheat and sunflower leaves. Aust J Plant Physiol **13:** 459–464
12. **Hinckley TM, Duhme F, Hinckley AR, Richter H** (1983) Drought relations of shrub species: Assessment of the mechanisms of drought resistance. Oecologia **59:** 344–350
13. **Kotcon JB, Rouse DI, Mitchell JE** (1985) Interactions of *Verticillium dahliae, Colletotrichum coccodes, Rhizoctonia solani,* and *Pratylenchus penetrans* in the early dying syndrome of Russet Burbank potatoes. Phytopathology **75:** 68–74
14. **Mathre DE** (1968) Photosynthetic activities of cotton plants infected with *Verticillium albo-atrum*. Phytopathology **58:** 137–141
15. **Nicot P, Rouse DI** (1987) Precision and bias of three quantitative soil assays for *Verticillium dahliae*. Phytopathology **77:** 875–881
16. **Pegg GF** (1989) Pathogenesis in vascular diseases of plants. *In* EC Tjamos, and CH Beckman, eds, Vascular Wilt Diseases of Plants: Basic Studies and Control. Springer-Verlag, New York, pp 51–94
17. **Pennypacker BW, Knievel DP, Leath KT, Pell EJ** (1989) Photosynthesis and stomatal response in a susceptible alfalfa clone infected with *Verticillium albo-atrum* (abstract). Phytopathology **79:** 1144
18. **Rowe RC, Davis JR, Powelson ML, Rouse DI** (1987) Potato early dying: Causal agents and management stategies. Plant Dis **71:** 482–489
19. **SAS User's Guide: Statistics, Version 5** (1985) SAS Institute, Inc., Cary, NC
20. **Sharkey TD** (1985) Photosynthesis in intact leaves of C_3 plants: Physics, physiology and rate limitations. Bot Rev **51:** 53–105
21. **Sharkey TD, Seemann JR** (1989) Mild water stress effects on carbon-reduction-cycle intermediates, ribulose bisphosphate carboxylase activity, and spatial homogeneity of photosynthesis in intact leaves. Plant Physiol **89:** 1060–1065
22. **Sharkey TD, Vassey TL** (1989) Low oxygen inhibition of photosynthesis is caused by inhibition of starch synthesis. Plant Physiol **90:** 385–387

23. **Terashima I, Wong S-C, Osmond CB, Farquhar GD** (1988) Characterization of non-uniform photosynthesis induced by abscisic acid in leaves having different mesophyll anatomies. Plant Cell Physiol **29:** 385–394
24. **Tzeng DD, DeVay JE** (1985) Physiological responses of *Gossypium hirsutum* L. to infection by defoliating and nondefoliating pathotypes of *Verticillium dahliae* Kleb. Physiol Plant Pathol **26:** 57–72
25. **Van Alfen NK** (1989) Reassessment of plant wilt toxins. Annu Rev Phytopathol **27:** 533–550
26. **von Caemmerer S, Farquhar GD** (1981) Some relationships between the biochemistry of photosynthesis and the gas exchange of leaves. Planta **153:** 376–387
27. **Vos J, Groenwold J** (1988) Water relations of potato leaves. I. Diurnal changes, gradients in the canopy, and effects of leaf-insertion number, cultivar and drought. Ann Bot **62:** 363–371